Sequencing a Genome:

Inside the Washington University Genome Sequencing Center

Activity Supplement

Paper PCR

(DNA Amplification)

Project Outline

The multimedia project *Sequencing a Genome: Inside the Washington University Genome Sequencing Center* is aimed at increasing the scientific literacy of biology students in the technology of genomic sequencing.

The following four video pieces are included on VHS cassette or CD:

- A guided tour of the Washington University Genome Sequencing Center, providing a look at the labs and offices that make up the preparation, production, and data management facilities. Includes animated explanations of the processes used to sequence genomic DNA.
- Exploration of current genomic research in pathogenic bacteria through an interview with a molecular microbiologist.
- Information about careers available at the Genome Sequencing Center presented through interviews with actual employees.
- An animated explanation of the chemistry of cycle sequencing using dideoxynucleotides.

Additional CD features include scripts of the video pieces, links to additional resources, and a glossary of terms.

As the scientific procedures presented in the video tour are complex, simple activities were specifically designed to better explain and reinforce the key concepts of restriction fragment mapping, PCR, sequencing, and electropherogram interpretation. The following paper modeling activity is an inexpensive and simple solution to presenting PCR.

Acknowledgments
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PAPER PCR
TEACHER MANUAL

Lesson Overview

Paper PCR is designed to provide your students with a deeper understanding of PCR and DNA amplification through readings, hands-on paper modeling, and completion of a worksheet. They will read about the required reaction components and thermal cycling and then model the actions of the components in a paper simulation of three cycles of PCR. Student pairs will play the part of *Taq* DNA polymerase, making several copies of a particular DNA sequence of interest. The whole class will represent several reactions occurring simultaneously in a single reaction tube. You will play the part of the PCR machine, directing the timed changes in temperatures during the three cycles of PCR. The students must blindly select one base at a time from a grab bag and put incorrect bases back into the grab bag as they go. The copy masters have sufficient components to model three cycles of PCR. The worksheet will ask them to explain the actions of the components and think about possible applications of PCR technology.

Paper PCR is specifically designed to precede Paper Terminators, as a fundamental understanding of PCR technology is critical to understanding PCR-based dye terminator sequencing.

Timeline

The background reading, paper modeling, and worksheet require 50 minutes to complete. The supplemental reading should only require 5 minutes and could be assigned as homework.

Materials

For each pair of students:

- 1 DNA Template (blue)
- 7 Forward Primers (green)
- 7 Reverse Primers (green)
- 1 Nucleotide Grab Bag (envelope) containing the following:
  - 36 As (white)
  - 36 Ts (white)
  - 36 Cs (white)
  - 36 Gs (white)
- 1 roll of transparent tape

Advance Preparation

- You will need to print and cut out one complete set of copy masters per pair of students. The paper pieces will be much more durable if they are laminated before they are cut out and lamination will ensure that you will be able to use the models year after year.
- Black print on white paper may be used, but using different colored papers will help students recognize the different components of the PCR reaction. If you choose to do

this, we suggest you print the DNA Template page on blue paper, the Forward Primer and Reverse Primer pages on green, and the Nucleotide pages on white.

- Cut out the DNA Template and tape it together as indicated. It should result in a 16 base pair long double-stranded fragment with the following nucleotide sequence:

  TAGTCACGCTAAGTCT
  ATCAGTGCGATTCAGA

- The double-stranded DNA Template will need to be separated into two complementary single strands before the activity can begin. You can choose to cut the DNA Template ahead of time, or have your students do this as a part of denaturing the DNA Template for the first cycle of PCR.

Hints and Troubleshooting

- You may find it necessary to demonstrate the paper modeling to the class before allowing the student pairs to work on their own. We suggest making overhead transparencies of the copy masters.
- If the DNA Template appears to be too long for your students to handle, the middle segment can be eliminated, resulting in the following 11 base pair fragment:

  TAGTCAAGTCT
  ATCAGTTCAGA

Supplemental Reading

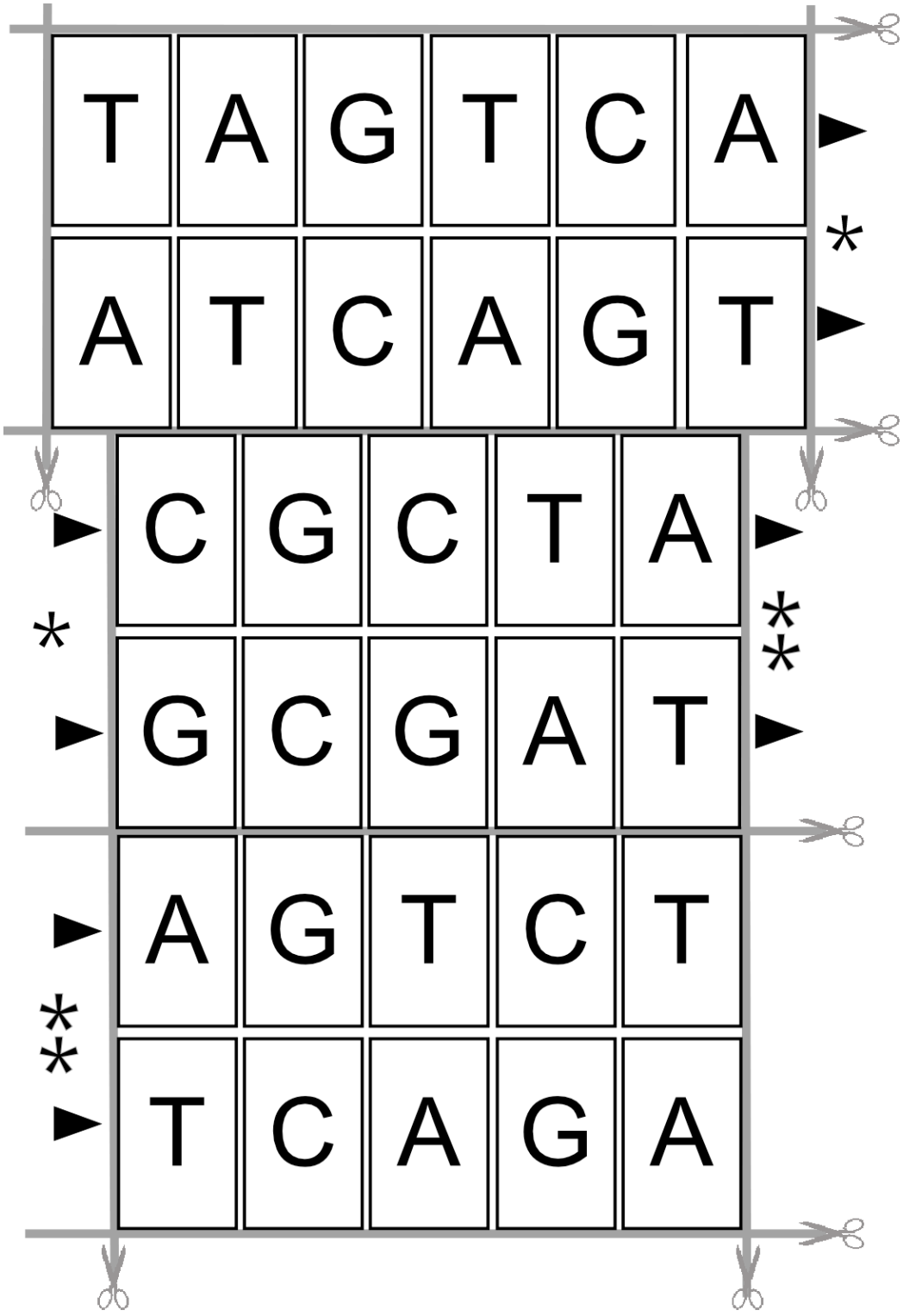
"PCR: The Accidental Discovery of Something Obvious"
Your World/Our World Volume 5, Issue No. 2, pages 12-13
http://www.biotechinstitute.org/yourworld.html

Answers to Student Worksheet

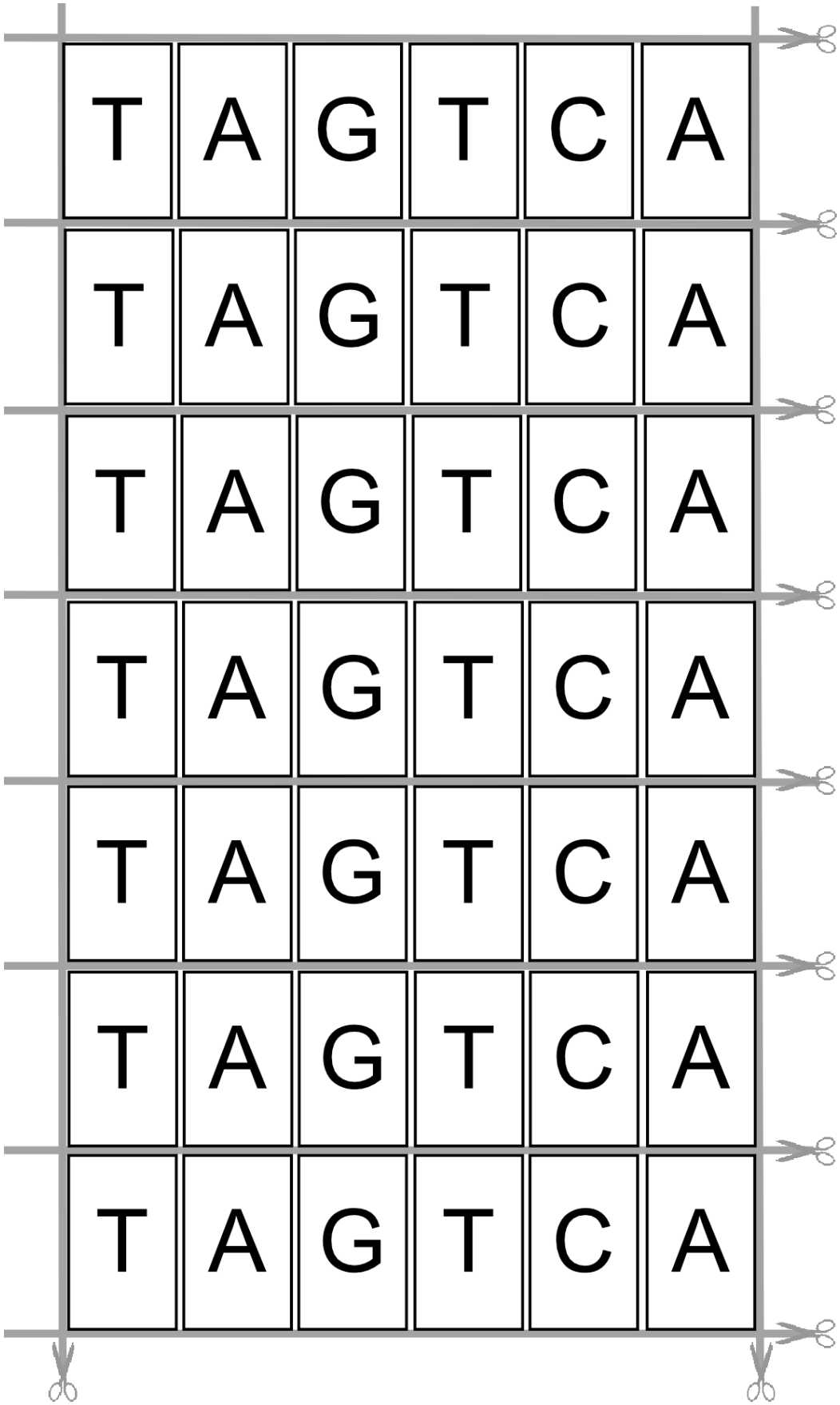
1. What are the four main components of a PCR DNA amplification reaction?
   DNA template, Taq DNA polymerase, oligonucleotide primers, and nucleotides
2. What is the name for the first step of thermal cycling? ___*denaturing*___
3. Describe what occurs during this first step.
   The reaction mixture is heated up to 96˚C so that the double-stranded DNA template denatures and becomes single-stranded.
4. What is the name for the second step of thermal cycling? ___*annealing*___
5. Describe what occurs during this second step.
   The reaction mixture is cooled down to 50˚C so that the oligonucleotide primers can base pair with, or anneal to, the DNA template.
6. What is the name for the third step of thermal cycling? ___*extension*___
7. Describe what occurs during this third step.
   The reaction mixture is warmed up to 60˚C, so Taq DNA polymerase can perform synthesis. Taq puts free-floating nucleotides into the correct places along the DNA template so that a new complementary strand of DNA is extended from the primer.
8. How is PCR technique like a chain letter?
   Every time a chain letter is sent out, the number of new chain letters generated increases exponentially. Every time a round of PCR is completed, the number of DNA copies generated increases exponentially.

9. Describe some beneficial uses of PCR technology.
   Possible answers may include identification of criminal suspects, paternity testing, or genetic disease diagnosis.

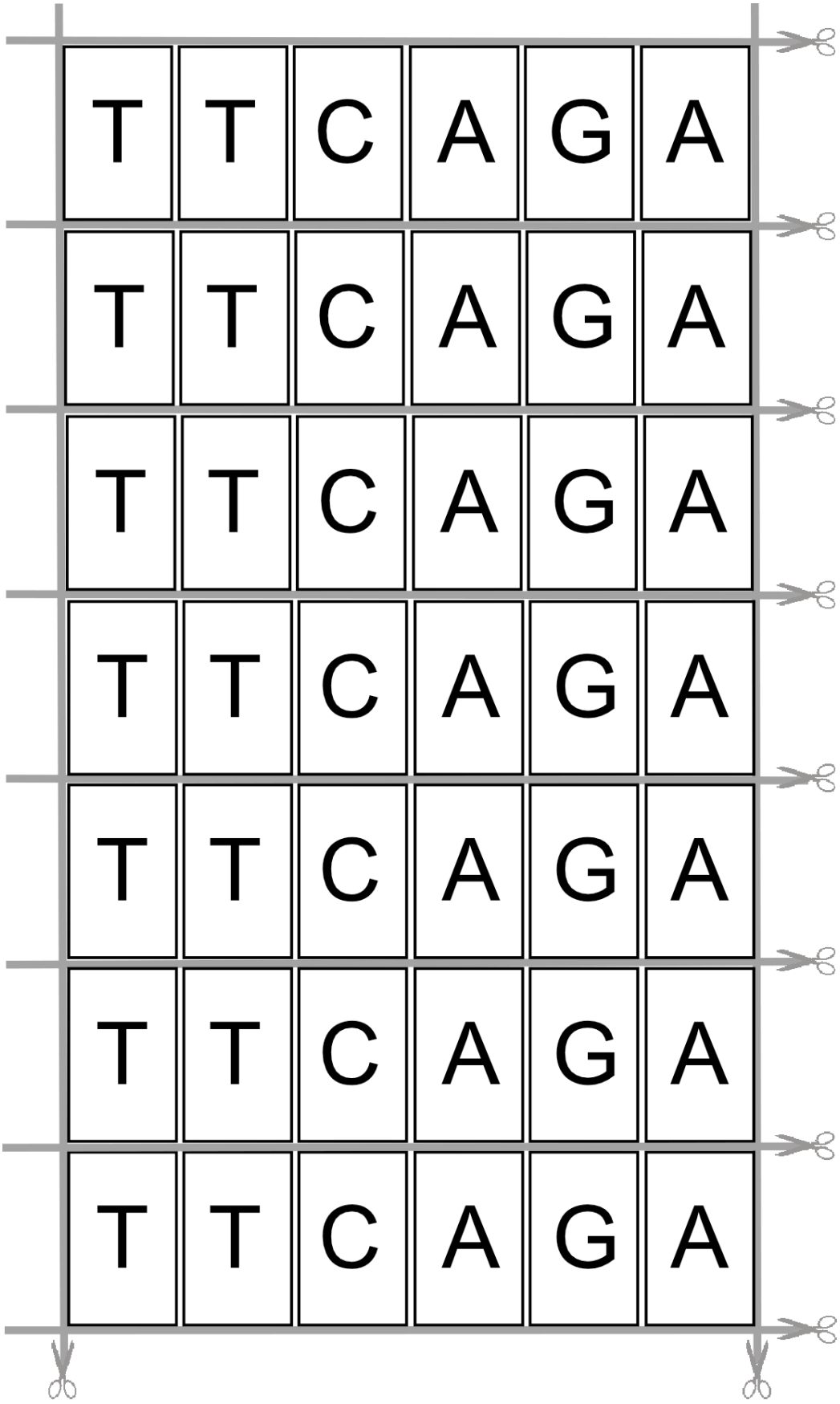
COPY MASTER
DNA Template (blue paper)



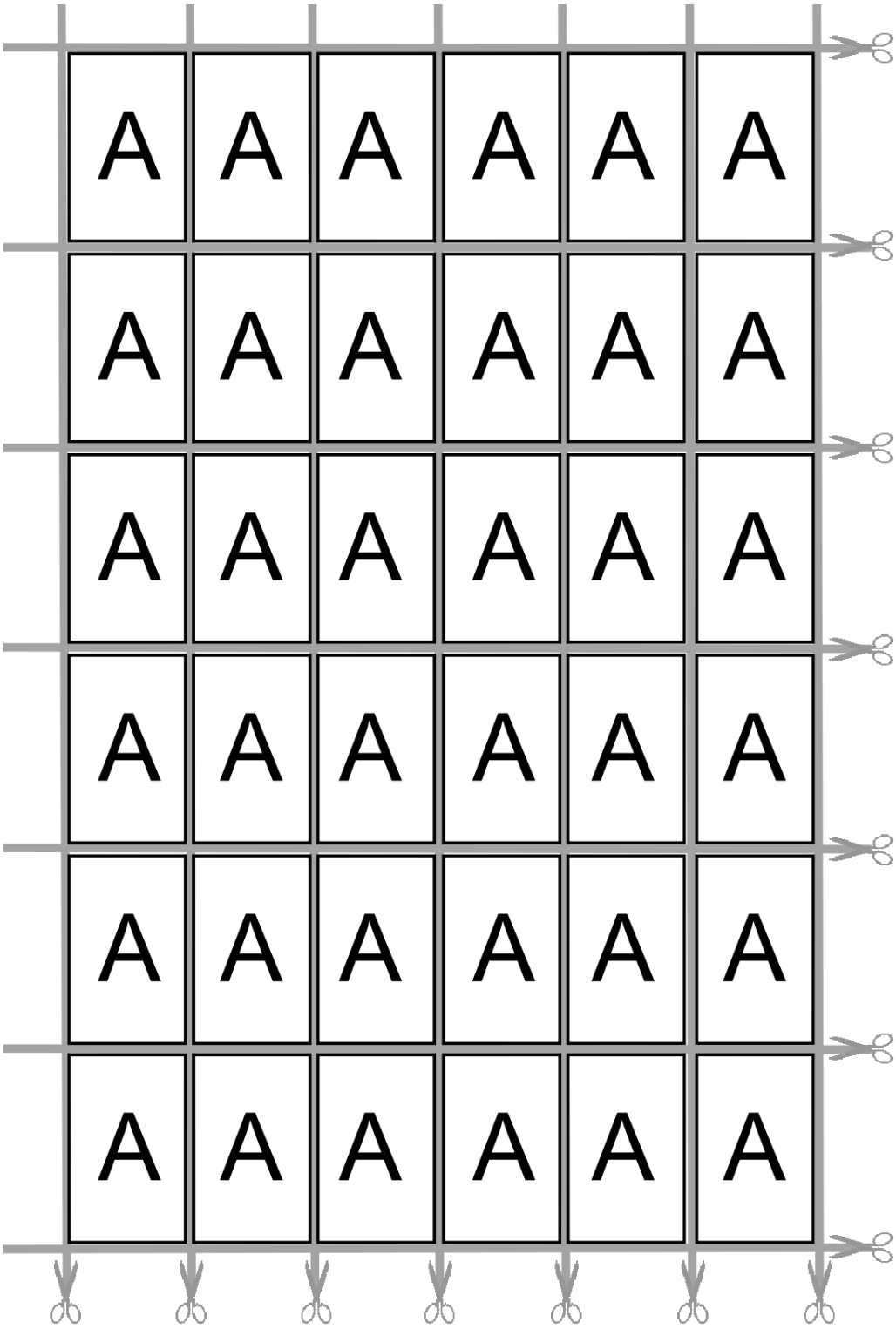
COPY MASTER
Forward Primer (green paper)



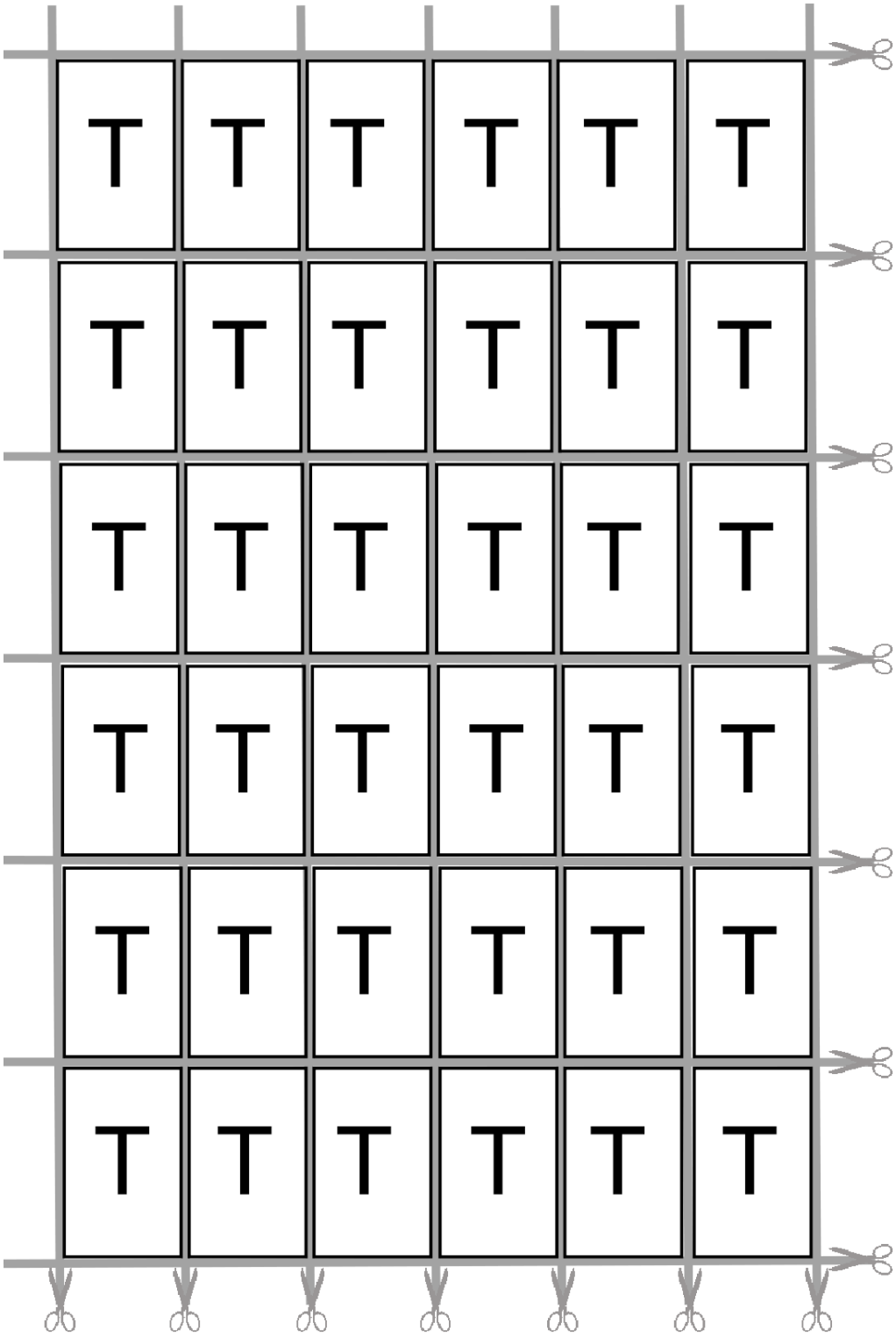
COPY MASTER
Reverse Primer (green paper)



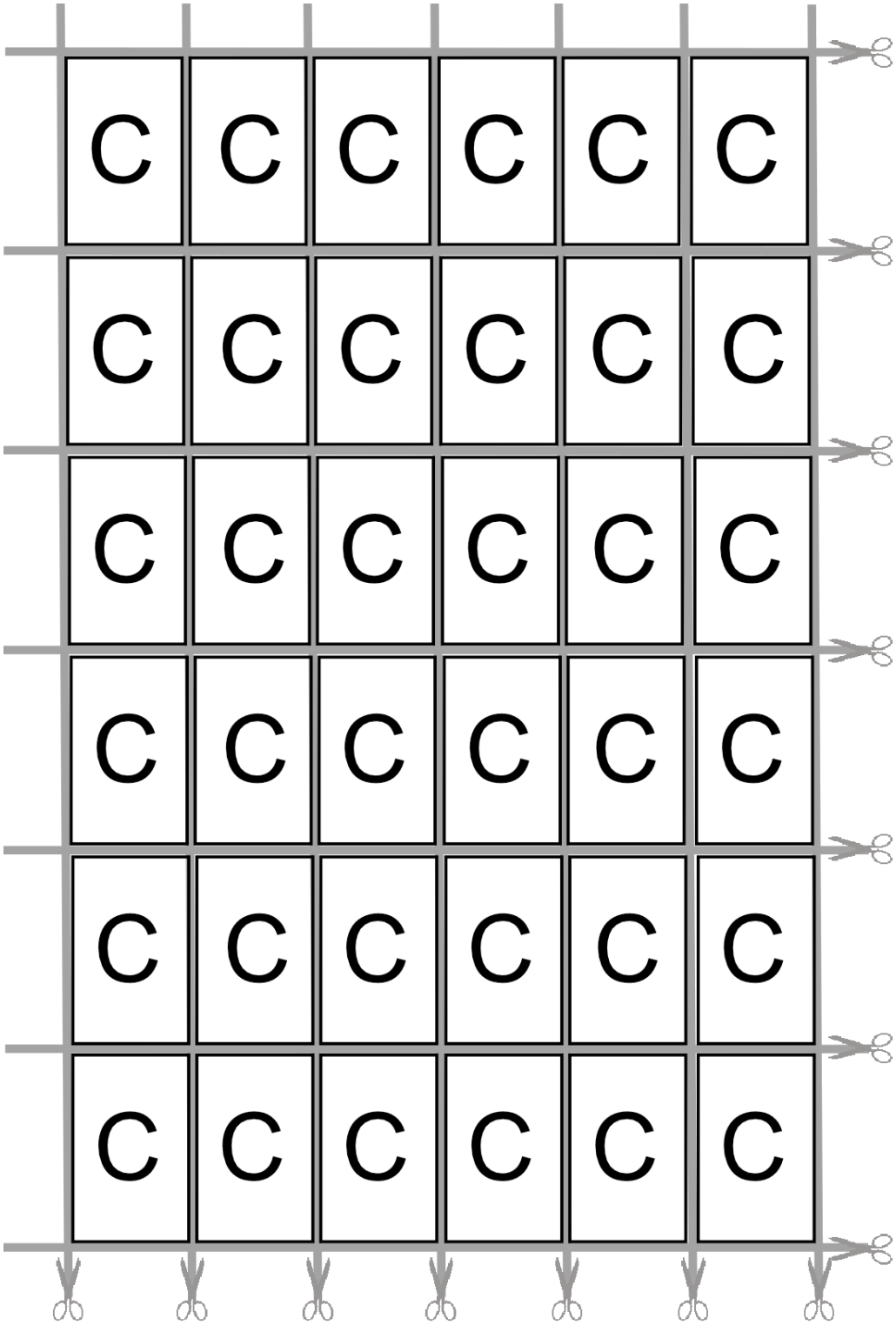
COPY MASTER
Nucleotide A (white paper)



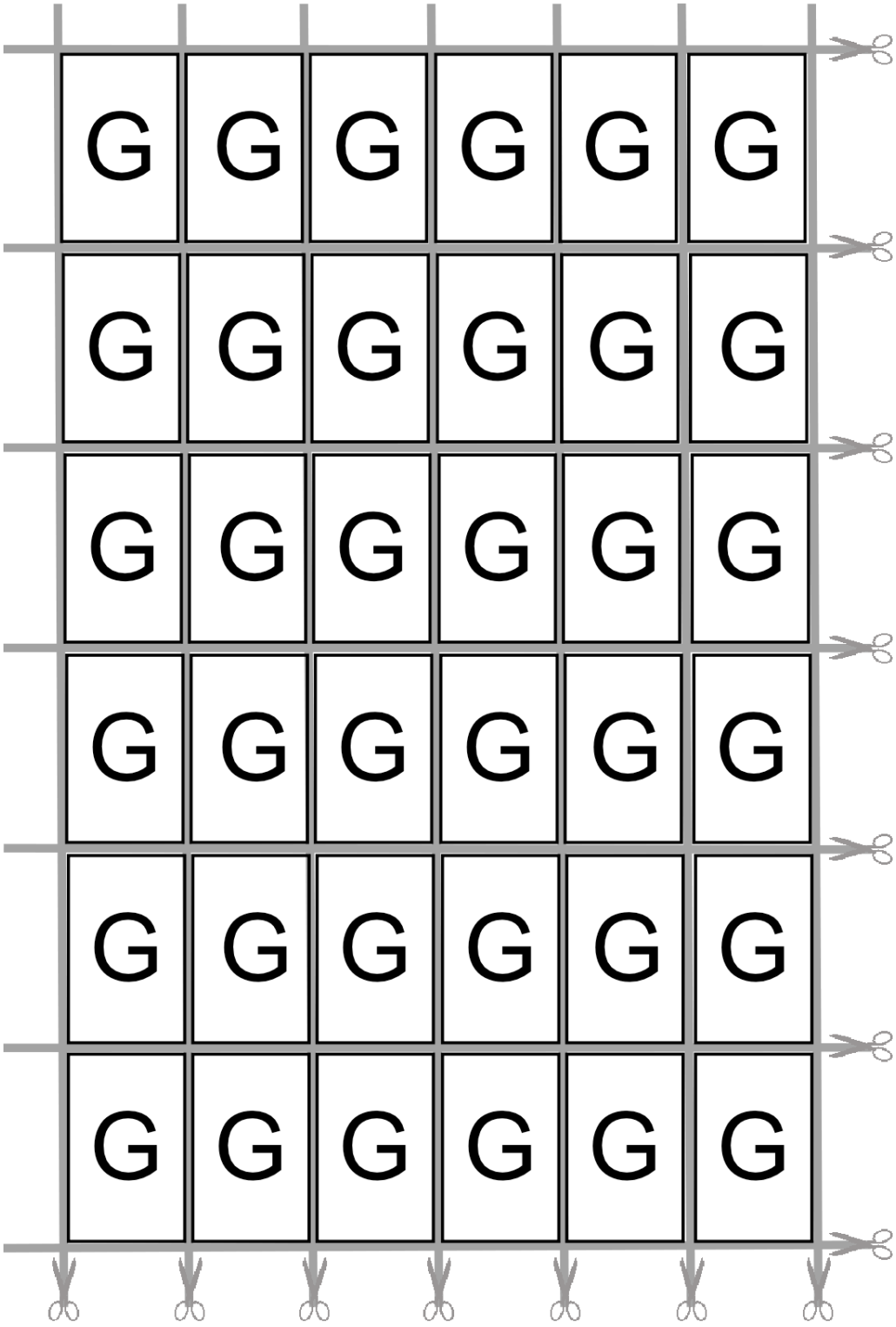
COPY MASTER
Nucleotide T (white paper)



COPY MASTER
Nucleotide C (white paper)



COPY MASTER
Nucleotide G (white paper)



PAPER PCR
STUDENT MANUAL

Background Information

The term **PCR** is an acronym that stands for the phrase **polymerase chain reaction**. PCR is a scientific technique used to amplify, or create millions of identical copies of, a particular DNA sequence, all within a tiny reaction tube. You can think of this procedure as similar to the DNA replication that occurs in your body cells every time they divide. However, PCR is different in two major ways. First, PCR only copies a specific region of DNA (rather than your entire genome). And second, it makes millions of copies of DNA (rather than just one).

PCR is an incredibly powerful tool that scientists use to analyze DNA. We refer to these kinds of reactions as DNA amplification reactions, although most people just call them PCR reactions. You may be most familiar with the use of PCR reactions in constructing human DNA profiles or "fingerprints" that can be used for forensics, paternity testing, and genetic disease diagnosis.

So, what things do you need to put into a tiny reaction tube to do a PCR-based DNA amplification reaction? Let's deal with each of the key components separately so you can understand the role that each one plays in the reaction.

DNA Template – This is double-stranded genomic DNA isolated from the cells of the organism being studied. It can be human DNA, plant DNA, mouse DNA, bacterial DNA, whatever DNA you would like to have copied! The point is that if you would like to copy something, then you must have a master template from which to start.

***Taq* DNA Polymerase** – This enzyme can add complementary nucleotides to a DNA strand during DNA synthesis. It is similar to the human DNA polymerase responsible for copying your genome every time one of your body cells divides.

Oligonucleotide Primers – These are short pieces of single-stranded DNA that match up to DNA sequences flanking (to either side of) the region of genomic DNA that you would like to copy. One is a forward primer and one is a reverse primer. When they have bound to the complementary sequences on the genomic DNA template strand, they show the *Taq* where to start DNA synthesis. The "oligos" or "primers" are responsible for making sure that only the region of interest is copied.

Nucleotides – Free floating single nucleotides must be present in the reaction because they are what the *Taq* puts in place during DNA synthesis. We can't copy DNA if we don't have something to make the copies with! So we must have As, Ts, Cs, and Gs in our reaction tube.

Unfortunately, we can't just micropipette all of these components into a tube and expect new DNA strands to magically synthesize. There is another critical part to PCR and this is the **thermal cycling**.

Specific changes in temperature, or thermal cycling, are what make the PCR DNA amplification reaction work. There are three different temperatures involved and a specific thing happens to the reaction components at each of those temperatures. Let's look at each one separately.

STEP 1 – DENATURING
The reaction mixture is heated up to 96˚C so that the double-stranded DNA template denatures and becomes single-stranded. (This high temperature breaks the hydrogen bonds between the complementary bases in double-stranded DNA.)

STEP 2 – ANNEALING
The reaction mixture is cooled down to 50˚C so that the oligonucleotide primers can base pair with, or anneal to, the DNA template. (This cooler temperature allows hydrogen bonds to form between complementary bases.)

STEP 3 – EXTENSION
The reaction mixture is warmed up to 60˚C, so *Taq* DNA polymerase can perform DNA synthesis. *Taq* can recognize an oligonucleotide primer as a starting point for DNA synthesis. It is able to put free-floating nucleotides into the correct places along the DNA template so that a new complementary strand of DNA is extended from the primer.

So, how do we get our tiny reaction tube to go through this series of temperatures? The tube is placed in a PCR machine, or thermal cycler, which is able to make rapid transitions between the different temperatures. Each time the reaction mixture is heated for denaturing, cooled for annealing, and warmed for extending, more DNA fragments are created. The reaction mixture in a single tiny tube can generate millions of copies of the region of interest. How is this possible? In the first round of PCR, only the initial genomic DNA serves as a template. However, in the second and all subsequent rounds of PCR, the newly synthesized copies can also be used as templates. This concept will become clear as you and your partner complete the Paper PCR modeling activity that follows.

<u>Activity Overview</u>

In this activity, you and a partner will play the part of *Taq* DNA polymerase as you amplify, or make several copies of, a particular DNA sequence of interest. Your whole class will represent several DNA amplification reactions occurring simultaneously in a single reaction tube. Your teacher will play the part of the PCR machine, directing the timed changes in temperatures during three cycles of PCR.

Materials

1 DNA Template (blue)
7 Forward Primers (green)
7 Reverse Primers (green)
1 Nucleotide Grab Bag (envelope) containing the following:
    36 As
    36 Ts
    36 Cs
    36 Gs
1 roll of transparent tape

Procedure

1. Locate all of the materials listed above. Lay out your double-stranded DNA Template in front of you and your partner. Wait for your teacher (the PCR machine) to start the first cycle of PCR.

2. Your teacher will indicate that the PCR machine has reached a temperature of 96˚C. You may now denature your DNA Template, or separate it into two single strands of DNA. Slide one strand in front of you and slide the complementary strand in front of your partner.

3. Your teacher will indicate that the PCR machine has dropped to a temperature of 50˚C. You and your partner may now anneal Oligonucleotide Primers to the complementary bases at each end of your single-stranded DNA Templates. One of you will be using a Forward Primer one of you will be using a Reverse Primer, depending upon which single-stranded DNA Template you have in front of you. (The Forward Primer will match up at the left end of one DNA Template and the Reverse Primer will match up on the right end of the other DNA Template.) Lay your Primer down so that the correct bases match up to those on your DNA Template.

4. Your teacher will indicate that the PCR machine has heated up to a temperature of 60˚C. You will now act as *Taq* DNA Polymerase, extending a complementary strand of DNA out from your Oligonucleotide Primer, one base at a time. To do this, you and your partner will each blindly choose a nucleotide from the Nucleotide Grab Bag.

    a. If your selected nucleotide does NOT correctly match up to the corresponding base on the DNA Template, then you must put it back in the Nucleotide Grab Bag and select again.
    b. If your selected nucleotide correctly matches up to the corresponding base on the DNA Template then you may lay it down and tape it to the end of the Primer.

5. Keep selecting nucleotides and putting them in place, taping each one to the previous one, until you have completed the whole double-stranded DNA fragment. Notice that

you and your partner now have identical double-stranded DNA fragments! You have used PCR to make an identical copy of your original DNA Template!

6. Your teacher will indicate that the PCR machine has once again reached a temperature of 96˚C. You may now denature your double-stranded DNA fragment. Notice that one strand is from the original DNA Template and one strand was newly synthesized in the first round of PCR. Both of these will be used as templates in the second round of PCR.

7. Your teacher will indicate that the PCR machine has dropped to a temperature of 50˚C. You may now anneal new Oligonucleotide Primers to the complementary bases at each end of your two single-stranded DNA Templates. One strand will require a Forward Primer and one will require a Reverse Primer.

8. Your teacher will indicate that the PCR machine has heated up to a temperature of 60˚C. You will again act as *Taq* DNA Polymerase, extending a complementary strand of DNA out from your Oligonucleotide Primer, one base at a time. It is going to get complicated, as you and your partner are each now synthesizing on two separate DNA Templates. When you have finished, you and your partner should have four identical copies of double-stranded DNA. The second round of PCR doubled your total number of DNA fragments again!

9. Follow your teacher's instructions through the temperature changes for a third cycle of PCR. When you have finished, you and your partner should have eight identical copies of double-stranded DNA in front of you.

10. Complete the Paper PCR Worksheet that follows.

11. Take apart all of your model pieces and return them to the Nucleotide Grab Bag.

Name _____ Class Hour _____ Date _____

Paper PCR Worksheet

1. What are the four main components of a PCR DNA amplification reaction?

2. What is the name for the first step of thermal cycling? _____
3. Describe what occurs during this first step.

4. What is the name for the second step of thermal cycling? _____
5. Describe what occurs during this second step.

6. What is the name for the third step of thermal cycling? _____
7. Describe what occurs during this third step.

8. How is PCR technique like a chain letter?

9. Describe some beneficial uses of PCR technology.